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FINAL REPORT

PREPARATIVE ELECTROPHORESIS EXPERIMENT DESIGN

Contract No. NAS8-28474

October 1972

Prepared for:

George C. Marshall Space Flight Center
National Aeronautics and Space Administration
Huntsville, Alabama

Beckman® INSTRUMENTS, INC.
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II

PREPARATIVE ELECTROPHORESIS EXPERIMENT DESIGN

SUMMARY

During the past eight months Beckman Instruments, Inc., has conducted a multifaceted study supporting the NASA programs to develop a space electrophoresis capability. The study involved principally the technique of continuous free electrophoresis. It comprised a critical review of the art, study of new techniques for enhancing resolution and stability, and construction and initial testing of a high-resolution cell. The effort resulted in a significant advance in free electrophoresis technique. It has provided also a much improved base for developments exploiting the added advantages of a zero-gravity environment. In the course of the program Beckman also proposed alternative space electrophoresis systems of a rotational type. It provided technical counsel in discussions with NASA, and participated in periodic review meetings. It provided practical assistance in the setup and operation of electrophoresis systems at MSFC and other facilities involved in the NASA program.

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1.0 INTRODUCTION

Electrophoresis is a powerful separative technique which, in numerous ways, has assisted the forward thrust of biochemistry, biomedical research, and applied medicine. One of the modifications of this technique--free electrophoresis--has occupied a distinctive and important position. By using a free liquid medium, thereby avoiding any supports such as gels, packed columns, and the like, this technique has been able to separate mixtures of large biological particles, e.g. whole living cells. In separating soluble components such as proteins, it has the advantage also of avoiding denaturation and loss which often accompany adsorption on a supporting matrix.

With elimination of the support, it becomes necessary to stabilize the liquid against convective disturbance in a different way. This has been variously accomplished with density gradients, periodic inversion or rotation of the medium, and the use of thin liquid layers subject to laminar flow. With any of these methods, however, the onset of thermal convection ultimately limits both the voltage which may be applied and the speed of separation.

It has been recognized that the availability of a zero-gravity environment offers a unique opportunity to enhance the performance of free electrophoresis. Convection, a gravity-dependent effect, disappears in this setting. Density gradients, which restrict the variety of particles which can be separated, become unnecessary. The tendency of large particles to settle, often a disturbing effect in earthbound electrophoresis, is likewise eliminated.

Beckman Instruments, Inc., was called upon by Marshall Space Flight Center to help assess and develop techniques implementing a free-space electrophoresis capability. Particular emphasis in this assignment was given to continuous free-flow electrophoresis, a technique in which Beckman is extensively

experienced and in which it played a major developmental role. In this report, we review the objectives of the program, summarize the accomplishments, and outline the needs for further study and development. Detailed technical data may be found in the monthly reports, dated April 12 to October 18, 1972.

2.0 OBJECTIVES OF THE PROGRAM

The objectives of the present program were the following:

- To review the state-of-the-art of continuous free electrophoresis, and to define areas of potential improvement.

- To study and modify the existing Beckman Continuous Particle Electrophoresis (CPE) system to optimize or improve performance in specified areas:

  Membranes. Examination of alternatives to present dialysis-type membranes (as separators between electrode and electrophoresis chambers).

  Cell Coatings. Evaluation of new materials to obtain better defined, more stable zeta potentials on inner cell walls, for improved control of resolution and stability.

  Deflection Capability. Improvement of resolution by increase of band deflection capability, by providing cooling at both cell faces, and increasing the length of the electric field zone.

- To support NASA in a general advisory capacity in electrophoresis and its applications in space, by participation in discussions, attendance at meetings, etc.

- To test candidate mixtures designated as potentially interesting for space electrophoresis, these mixtures to be assessed for quality of separation in earth gravity and potential sample-handling problems.

These objectives were met and reported upon, with the exception of the study of candidate mixtures. Due to modification of NASA's schedules, Beckman was advised that candidate mixture studies would be deferred beyond the present program period. Funding previously allocated for such studies was therefore, with approval of MSFC, distributed as additional effort on the other defined tasks.

3.0 SUMMARY OF ACCOMPLISHMENTS

3.1 Review of the State-of-the-Art

Our first report, April 12, 1972, included a "CPE Technology Status Summary." It outlined the history of free-flow electrophoresis as represented by earlier developments at the Max Planck Institute and Beckman Instruments, Inc. It highlighted the state of the art existing at the beginning of the program. Briefly, Beckman had developed a rapid-flow system optimized specifically for separation of particles. Most important, it found that the state of zeta potential (z.p.) of the cell wall dominated every significant aspect of the performance: resolution, sample throughput, and stability. The z.p. could be adjusted by means of easily applied, easily removed adhesive films and coatings.

Desired values could be obtained by an averaging effect in composite coatings applied to separate areas. A novel optical accessory made visible the cross-sectional shape of component bands as they passed through the CPE. This directly indicated the magnitude and sign of change needed in the cell wall z.p. to optimize resolution. It also provided various visual clues as to changes needed in operating conditions to avoid artifact bands and instabilities.

The Technology Status Summary discussed also the current limitations in CPE technique. Although resolution observed in the CPE cell was excellent, finely resolved bands were often so closely spaced that their collection in separate external vessels was difficult. A hydraulic fanning technique was proposed for increasing the separation. Also, for the higher resolution still needed for biological components, it was felt that a two-to-three-fold longer cell was required. New stable coatings of relatively low z.p. value were needed

for optimum focusing of biological component bands. Finally, superior membrane materials were needed for separation of the electrode channels from the curtain space. Specifically, membranes were needed which caused less change in the curtain pH. This requirement was felt to be especially important for the longer cells which we proposed building.

3.2 Membrane Studies

The membranes in the CPE provide a special form of barrier between the electrophoresis space and electrode chambers. They prevent appreciable mass flow between these spaces while providing ionic continuity between them. Prior to the present program, we used cellulose membranes exclusively, of the type usually employed in dialysis. These had several shortcomings. They caused a pH change in the curtain, particularly in zones bordering the membranes at either side. This arose from the fact that the transference numbers of the buffer ions (a measure of the proportion of current carried by each ion) had different values within the membrane and in the free buffer. This altered the ionic composition and pH immediately adjacent to the membranes, the change being then carried into the main cell space by electrphoretic action. In addition, the physical properties of the membranes (low visibility and lack of rigidity) made them very difficult to install in the cell. Due also to softness of the membrane, pressure fluctuations in the electrode chambers were very easily transmitted to the electrophoresis space, thus affecting the stability of the bands.

It was necessary to seek alternative, low-resistance materials of greater stiffness and superior ion transport properties. Low resistance was needed to minimize electrical heating within the membrane, a potential source of convective disturbance in the adjacent curtain zones. Materials which appeared especially promising were ion exchange membranes and microporous membranes. The use of ion exchange membranes had been cited by K. Hannig of the Max Planck Institute and claimed to be useful in minimizing pH effects. Microporous membranes had not, to our knowledge, been used before. Given the pore diameters of these membranes, many times larger than typical hydrated ion diameters, it appeared that ion sorting effects would be minimized.

Further, mechanical and handling properties of these membranes were far superior to those of dialysis membranes. In the design of the membrane tests, we planned as a basis of comparison to make quantitative measurements on the dialysis membranes.

3.2.1 Membrane Test Apparatus

It was felt at first that the membranes could be tested more expeditiously in a small special-purpose cell than in a CPE system. This would also facilitate tests with small available samples of candidate membrane materials, whereas the CPE required nearly foot-long strips. The test cell is described in our report of April 12, 1972. A disc of the membrane under test formed a divider between two small, buffer-filled spaces. Each space was coupled to an electrode chamber via a fritted glass disc, the latter being of a coarse structure which would not, per se, contribute a pH effect. The setup would permit making membrane resistance measurements; also, the chambers adjacent to the membrane could be sampled for pH change.

The resistance of the dialysis membranes in test cell proved to be surprisingly low, in fact low enough compared with the resistance of the total cell (filled with 0.01 M veronal buffer) that it could not be accurately measured. This suggested that convection effects in the CPE could be attributed mainly to the membrane-channel resistance rather than resistance in the membrane itself. This altered view minimized the need for further resistance testing of the membrane, especially since in initial rough measurements the microporous and ion exchange membranes appeared of even lower resistance than the dialysis membrane.

In our tests for pH change adjacent to the membranes, applied voltages varied from 150 to 500 V. Membrane current densities ranged to 3.0 mA/cm^2 of membrane, with test periods as long as 50 minutes. Only the highest current densities, comparable with values occurring in the CPE, gave significant pH change. For various reasons, however, the results were not found indicative of buffer changes actually occurring close to the membranes in the CPE. Thus, due to physical limitations, the resistance of the ionic path in the test cell

was quite high. This prevented use of typical low-molarity CPE buffers; e.g., 0.005 M or less. High-buffer molarity, on the other hand, tended to suppress any pH change. We realized, too, that laminar flow next to the membranes in the CPE created a much different situation from that in the test cell, where convective mixing could occur relatively freely. In the CPE, relatively sharp and stable pH changes could occur close to the membrane, due to the combined selective transference effects and absence of convective mixing.

3.2.2 In-Situ Membrane Tests

Due to these difficulties in using the test cell, it was decided that further membrane testing would be done in an actual CPE unit. Two membrane performance criteria were of interest: deflection stability observed with a polystyrene latex band, and uniformity of lateral pH distribution seen in effluent taken from the cell. Observations on dialysis membranes provided a performance baseline.

Band Stability. In past CPE experience, we commonly observed a lateral band instability of ±0.1 to 0.2 mm with an average period of several seconds. At times the instability was several-fold higher. Since our cells have varied in design, and membranes have differed at times in age and properties, the sources of instability could not be clearly pinpointed. A suspected source was electrical heating in the membrane. Heated buffer at the membranes could rise up out of the electrode slots irregularly, disturbing the lateral curtain stability. In extreme cases, warm buffer might rise along the curtain gasket edges into the upper neck of the gasket, where the effect on the curtain flow lines could be greatly exaggerated. Diaphragm action of the membrane, dependent on membrane softness and caused by pressure fluctuations in the rinse channel, could also affect band stability.

Since these instabilities could vary with membrane properties, it was desirable to observe the stability obtained with various membranes under controlled conditions. Our reference tests were made with standard (double layer) dialysis membranes. Using 0.015 M veronal, electrode rinse at 3°C, and cooling bath at -10°C, we were surprised to observe excellent band position stability

(±0.1 mm) at a total wattage dissipation in the cell of 135 W. Testing at higher wattages was limited only by the capacity of the circulating cooler. In tests at lower molarities (down to 0.001 M) using increasing voltages, similar band stability was seen up to the 600 V limit of the power supply. Apparently, then, occasional instabilities greater than ±0.1 were caused not by resistance of the dialysis membrane per se, but to occasional sub-optimal cooling of the curtain or electrode rinse, change in membrane stiffness with age, etc.

Band stability was similarly tested with ion exchange membranes (Ionics, Inc., cationic 61 A2L183 and anionic 111B2L183, at anode and cathode, respectively), and with a variety of microporous membranes. With both the ion exchange membranes and a 0.22 micrometer pore filter membrane (Millipore HA, mixed cellulose ester), bands were stable up to about 50 W total heat dissipation. In another test on a microporous membrane, with an apparent slight continuous flow of buffer through the membrane, stability was seen up to the 70 W level, the applied voltage, 600 V, being the power supply maximum. This observation was made on a 0.10 micrometer HA membrane, the pressure in the rinse channel being deliberately lowered to give a slow outward flux of buffer from the curtain.

The stabilizing effect of flow through the membrane may be explained as follows: buffer within the electrode slots is heated electrically, due both to buffer resistance in the slot and resistance in the membrane. Outflow from the cell space to the rinse channel via the membrane removes this warm layer before it attains a temperature giving convective disturbance.

pH Effects. In these in-situ membrane tests, we measured the lateral pH distribution in the curtain as seen in cell effluent taken from the collector tubes. The ion exchange membranes (Ionics, Inc., cited above) performed relatively poorly. Microporous membranes, on the other hand, more than fulfilled our expectations; they gave a nearly uniform pH across the entire curtain width.

The pH measurements were made after 30 minutes equilibration with buffer flowing and voltage continuously applied. This assured equilibration of the very slow-flowing layer adjacent to the membrane surface. Measurements were made with veronal pH 8.6 buffer of various molarities and with various voltage gradients. Where membranes showed a tendency to alter the curtain pH, the effect was consistently greater at lower molarity, due to diminished buffering action, and consistently greater with increase of the voltage gradient.

Measurements were first made with standard (double-layer) dialysis membranes to provide a basis for comparison. The results confirmed observations made early in the CPE development program. The effluent showed a pH increase of about 0.3 pH units close to the anodic membrane, almost independent of the molarity. An elevation of pH was also seen about 1 cm from the cathodic membrane, attaining 0.3 pH units elevation in .001 M buffer at 90 V/cm voltage gradient. These pH changes have not been serious in the present cell with its 40-cm-long field zone. However, in the longer cell we were to build later, with proportionately greater penetration of the pH change into the curtain, these disturbances could be quite troublesome.

The ion exchange membranes (Ionics, Inc., see above) were typical commercial membranes of low electrical resistance. The cationic membrane was applied next to the anode, the anionic membrane next to the cathode. With .001 M buffer, the curtain pH deviated from the original pH 8.6 value across the total cell width. Values ranged from pH 11.2 at the cathodic side to 7.8 at the anodic side. With .005 M buffer, the pH was unchanged in effluent from tubes 12 to 45 (in a total of 48, counting from the cathodic side), but in tubes 1 to 6 varied from pH 11.1 to 9.3.

The microporous membranes tested included Tyrann II (Chemical Systems, Inc., 3.0 micrometer pore size) and Millipore type HA filter membranes with pore diameters of 0.45, 0.22, and 1.0 micrometers. Ideal behavior was observed when we tested a double layer of the 0.22 micrometer pore membrane. The pH across the full curtain width was uniform within the precision of measurement (±0.01 pH units). A single 0.22 micrometer membrane was only slightly less

perfect, showing a 0.1 pH drop closely adjacent to the cathode. With the more porous membranes such as Tyrann II and the HA 0.45 micrometer, there were somewhat larger pH departures at the curtain edges. The results here may have been clouded, however, by slight flow of the electrode rinse buffer into the curtain via the membranes. In a test with 0.1 micrometer pore HA membrane, in which we deliberately lowered the pressure in the rinse chamber, we again found the curtain to be uniform within 0.05 pH units across its total width.

The microporous membranes represent a significant advance in CPE technique. They have nearly ideal ion-transfer properties, and are mechanically far superior to the dialysis membranes. When wet, they have good resistance against tearing. Also, being more visible and stiffer than the dialysis membrane, they are much easier to handle and install in the CPE cell.

3.3 Cell Coating Studies

The surface properties of the internal cell faces are, as stated earlier, of controlling importance in free-flow electrophoresis. The surfaces must have a uniform z.p. value, and for optimum resolution must be adjusted in value according to the electrophoretic mobility of the bands of greatest interest.

In simplified terms, any band of particles traversing the cell has two significant components of motion--one vertical, the other horizontal. Both of these velocities have a range of values, depending in a defined manner on the range of depth occupied by the particles in the curtain thickness. These velocity variations cause a broadening of the bands and loss of resolution. Under a unique condition, however, the broadening of any band can be nullified and the band "focused" by a mutual compensation of the vertical and horizontal velocity variations. This occurs at a value of wall z.p. appropriate to each particular band to be focused. For particles which are "large," electrophoretically speaking, the wall ideally has the same z.p. as the particle. The theory of this free-electrophoresis focusing phenomenon has been developed in detail and described in publications (references cited, report of April 12, 1972).

3.3.1 Evaluation of Coatings

It was essential to develop several z.p.-stable coating materials, with appropriate application techniques, to cover a useful z.p. range. Intermediate values could be obtained by an averaging process, using pairs of coatings of different z.p. values. In our first experiments, we measured the relative z.p.'s of three materials already familiar to us: Mylar, collodion, and gelatin. As with all other coatings used, these were negatively charged. They had respectively high, medium, and low z.p. values. The Mylar and collodion were relatively unstable in z.p., the value diminishing steadily after installation of a fresh coating. This was found to be due to adsorption of contaminants from the buffer by these relatively hydrophobic surfaces. The source of the contaminant was mainly the PVC tubing in the curtain buffer recirculation system. When this was replaced by glass tubing with short silicone rubber connections, the stability of the cell wall z.p. was considerably improved. Gelatin coatings were found to be relatively stable in tests lasting several days. Unfortunately this material is too biodegradable for more than short-term use. When we tested Lexan (polycarbonate resin, General Electric) at NASA's request, it was found to have the same z.p. as collodion.

We next examined hydrophilic coating materials which might provide both stability (in virtue of low adsorption tendency) and, as a group, a relatively wide range of z.p. values. "Hydron" hydrophilic polymer (Hydron Laboratories) was found to give a relatively high z.p. approaching that of Mylar. Agarose was of low z.p. value as expected, somewhat above that of gelatin. Ionagar (Colab) gave a z.p. half-way between that of agarose and collodion. We obtained a sample of caragenan, a seaweed-derived gelling agent related to agarose, and made arrangements to secure some agaropectin, the ionic component of natural agar. These substances are expected to provide a relatively high z.p. combined with low biodegradability. Both these materials remain to be tested.

In anticipation of continued work on coatings, we have made a further study of the literature on substances of low z.p. value. This included now also a search for low z.p. reference particles. These are needed for visualizing

the focus when the sample bands per se are of low z.p. value, and are either invisible or diffuse in their mobility.

Proteins are an interesting, potentially valuable wall coating material. Gamma globulins, for example, typically have low mobility, are readily obtainable commercially, and easily preserved. Techniques are available for coating them on solid surfaces. As a complementary reference particle, gamma globulin-coated polystyrene latex is commercially available. Gelatin coatings, preserved by any of a variety of hardening processes, may be useful. Another promising category is that of ion-exchange resins. In dried, brittle form, a mixed anionic-cationic resin of a low total charge could be ground to a fine powder and layered on a water-insoluble pressure-sensitive adhesive substrate. The powder itself could serve as a reference sample material. Insoluble polysaccharides such as starch and cellulose are of interest. Cellulose surfaces could be generated in situ by surface hydrolysis of cellulose ester coatings. Commercially available microcrystalline cellulose powders may serve as reference samples. Evaluation of these substances and development of appropriate coating techniques will assist significantly the biological application of CPE technique.

In our work with agar and agarose, we had to develop techniques for adhering these to the cell walls. They would not directly adhere to acrylic or glass surfaces, nor to Mylar film, either uncoated or precoated with collodion. They were successfully applied, however, to Dupont "Cronar" polyester film, prepared by the manufacturer with a "subbing" layer. The latter, intended for adhesion of photographic emulsions, permitted adhesion of the agarose and agar as well. The Cronar was attached to the cell with a thin layer of pressure-sensitive adhesive.

3.3.2 Sample and Coating Correlation Studies

We had little prior experience in applying the focusing technique to bands of biological materials. We expected these substances to be generally of lower zeta potential than the polystyrene latex range. Proteins would be of special interest among biological components, whether as soluble components in a

mixture or as a common constituent of cell surfaces. It was of interest, therefore, to know how the range of protein mobilities related to that of polystyrene latex. This would determine which coating materials would be most effective in focusing bands of proteins or proteinaceous particles.

Serum gamma globulins are known to have near-zero mobility in typical alkaline buffers. Serum albumin, on the other hand, exhibits a near-maximum among protein mobilities. We measured in the CPE the relative electrophoretic displacements of bromphenol blue-stained albumin, various naturally colored proteins, and a family of polystyrene particles. A chart was constructed in which the "osmobility" characteristic of various wall materials was correlated with the electrophoretic mobility of the various sample materials. The osmobility is a measure of the electro-osmotic mobility of the buffer layer next to the cell wall, and is proportional to the wall z.p. Osmobility was plotted on the ordinate axis, sample mobility of the abscissa axis. With equal scaling of the axes, a 45-degree line drawn from the origin represented a line of focus. It showed which wall material would provide optimum focusing of any sample material. Or, in a dual-coated cell, it indicated the necessary proportions of two differently coated areas which would provide a focus. The chart, shown in the report of July 17, 1972, presents data on all the coatings mentioned above as well as the following sample materials: polystyrene latex (PSL) 0.109 to 1.011 micrometer particle size; agarose-coated 0.50 micrometer PSL; bovine albumin, horse spleen ferritin, and beef liver catalase.

3.3.3 Foucault-Test Viewer

As an aid in making mobility measurements, particularly on otherwise invisible protein bands, we devised a Foucault viewing system. The Foucault test is well known in mirror and lens testing. It detects departures from a spherical wavefront due to refractive or surface irregularities in the test object. The light from a point source is collimated, directed through the test object, then refocused. The operator, viewing the test field from a position just behind the focal point, inserts an adjustable knife edge laterally into the beam at the focus. Defects in the test object show up as apparent

three-dimensional relief in the object field. We were able with this viewing system to detect bands of albumin in the CPE cell at a concentration of less than 0.1 percent.

3.3.4 Electrical Focusing

Having improved the stability of the Mylar and collodion coatings by using a high-purity buffer supply, we were in a position to test further an advanced focusing technique conceived earlier at Beckman. This consisted in an electrical averaging of the z.p.'s of two different coatings applied to upper and lower halves of the cell. Each half of the cell was provided with a separate pair of electrodes, each pair being powered by a separately adjustable voltage supply. With different voltage gradients applied at the two coatings, the effective z.p. of the cell was the average of the separate z.p. values, each weighted by its relative voltage gradient. Accordingly, any desired band in a spectrum of separated components could be focused simply by turning a knob which varied the voltage ratio. A special cross-sectional illuminator, revealing the cross-sectional shapes of bands, served as a "null meter" during focusing.

We demonstrated and documented the electrical focusing principle using a dual coating of Mylar and collodion and a four-component polystyrene latex sample. Each band was successively brought to focus by progressive change of the ratio of voltages applied to the upper and lower coatings. The focusing of the bands and the extent of band shift accompanying the operation were precisely as predicted by theory. A paper on the focusing theory, and a demonstration of the effect with a series of photographs, was presented at the New York Academy of Sciences International Conference on Isoelectric Focusing and Isotachophoresis, May 19, 1972. The paper will appear shortly in the Annals of the Academy.

3.4 Improved Cell Design and Construction

Prior to the present program, a background of CPE theory and experience had accumulated during several years of use of the system. This knowledge had yet to be applied to a new, more effective design. A new quantum jump in the effectiveness of the technique now seemed possible. Importantly, an updated

capability would better define for NASA the status and limitations of continuous electrophoresis as experienced in earth gravity.

Several improvements appeared necessary and feasible. To increase the resolution, especially for low-mobility biological particles, the effective field length had to be increased two- to three-fold. We wished, in addition, to expand or laterally magnify the pattern of separated bands as it emerged from the cell. This would permit convenient external collection of finely resolved components. We needed to cool both faces of the flowing curtain, rather than one face only. This would double the wattage which could be dissipated in the curtain without excessive heating of sensitive biological samples. Higher levels of buffer concentration could then be used. The cell would have three pairs of electrodes, applicable to three coated areas in the cell, permitting both focusing and compensation of cell-wall asymmetry (i.e., difference in z.p. between front and rear wall surfaces). Since the date of the last monthly report, the new long path cell has been completed and subject to preliminary testing. In a separation of mixed polystyrene latex, it performed in all respects as predicted.

The cell was fabricated in methyl methacrylate (Plexiglas) for ease of machining and resistance to cracking, in preference to polycarbonate resin. The cell provides an 80-cm-long electric field, twice that of the previous CPE systems. The curtain is 7.5 cm wide, half again the former width. There are three pairs of electrodes. One of these flanks the upper half of the cell; each of the other pairs flanks a quarter of the remaining height. Varying the ratio of the voltages applied to the two shorter sections compensates for asymmetry. For focusing, this ratio is held constant while the gradient applied at the upper half of the cell, relative to the average of the two other voltages, is varied.

The cooling chambers, in both front and rear plates, are of new design. The cover plates are now made of Plexiglas rather than glass. This will eliminate breakage caused by thermal expansion differences. Flow within each cooling chamber is lateral rather than longitudinal. This shortens the transit time

of the coolant through the cell, reduces pressure drop in the cooling space, and increases coolant flow. The vertical temperature gradient in the curtain is virtually eliminated. The closely spaced horizontal ribs in the cooling chamber act both to direct the flow horizontally and to rigidize the cover plate. The cover plate may accordingly be made quite thin for more effective cooling, yet not exhibit excessive diaphragm effect due to pressure fluctuations in the coolant.

A new sealing arrangement is used for the curtain flow space. Two opposed O-rings now seal opposite faces of the curtain gasket. Thus, the thickness of the curtain can be changed at will simply by changing the gasket thickness. In the past, it has been very difficult to study this variable in cell design.

A fanned-out lower section of the cell expands the curtain width two-fold to 15 cm. Only a third of this total width (sufficient for collecting any actual set of separated components) is fitted with collector tubes. These tubes, 40 in number, occupy a zone somewhat to the right of center. The rest of the curtain width provides clearance at the two sides, and room for the electro-osmotic deflection component. The portions of the curtain not passing through the tubes drain through a common manifold via an adjustable valve.

In a preliminary test of the cell, without coolant flow, we separated a mixture of 0.109 and 0.481 micrometer diameter polystyrene latex particles. Using .001 M buffer, 21 ml/min buffer flow and 280 volts applied to each of the three electrode pairs, separation of the bands before entering the expander was 5.0 mm. The bands were sharply defined and undistorted. After passage through the expander, the separation was 10.0 mm. This is a several-fold larger separation than ever seen previously for these components. Only the limited width of the cell prevented use of higher voltages to increase the separation further. This will not be a limitation, of course, when biological materials, typically of lower mobility, are used.

The full capabilities of the cell will be assessed later when it has been appropriately coated, tested for focusing and asymmetry compensation, and applied to the separation of biological samples.

3.5 Supplementary Studies

In our advisory capacity to MSFC, we studied the possibility of alternative space electrophoresis sytems. Such systems might offer an intermediate capability between that tested in the Apollo missions, and the proposed continuous electrophoresis system. We described certain forms of rotary electrophoresis which might satisfy such a requirement.

A limitation of the Apollo design was the difficulty of localizing the sample initially in a narrow band. Another limitation of such a system was that separated fractions would not be removable from the cell without loss of resolution. A means for stabilizing the liquid during these operations appeared essential. We proposed an improvement consisting of rotating the liquid, for example by means of a cylindrical rod rotated coaxially in the cell. Our experience in the CPE suggested that shear effect within the liquid annulus would stabilize the liquid. This would permit injection and localization of the sample as a thin ring. The fractions, also ring shaped, would be collected by a movable tip, or by a fixed tip intercepting successive fractions as they reached the pickup position.

We performed several experiments to test the hydrodynamic behavior of such an arrangement. It was found that centrifugal effects could set up circulating cells in the annulus, somewhat akin to the Benard convection cells observed in thin liquid layers heated from below. In analogy to the thermal case, it may be possible to suppress such cells by limiting the thickness of the annulus and the rotational velocity. An alternative is to rotate the cylindrical wall of the cell while holding the core stationary. Our experiments showed that the latter arrangement did indeed suppress circulation cell formation. However, our relatively crude cell introduced some longitudinal motion in the liquid, tending to broaden the bands. These difficulties do not appear unsurmountable, especially since the rotation may be confined to the initial and terminal stages of the run, during sample introduction and fraction collection. We believe that rotary electrophoresis remains an interesting possibility, worthy of further investigation.

3.6 Miscellaneous

Several cross-country trips were made by Beckman personnel. Two trips were made to the General Electric Space Sciences Laboratory (Valley Forge), and two trips to the Marshall Space Flight Center. Meetings at GE were devoted to a review of proposed continuous electrophoresis experiments. Meetings at MSFC included a review of status of the space electrophoresis program and discussions of alternative space electrophoresis systems. In separate trips to GE and MSFC, assistance was given in setting up and operating CPE units at both these locations.

4.0 PROPOSED FURTHER STUDIES

A continuation of the present program, consolidating the gains of the past eight months, will contribute significantly to NASA's preparation for a space electrophoresis capability. It will apply newly developed theory and techniques to establish clearly the critical operating parameters of free electrophoresis. It will define the limitations of this separation technique in the environment of earth gravity. It will optimize free electrophoresis in the terrestrial setting in preparation for further important potential gains in the zero gravity of space.

The present effort must be continued along several lines: completion of the system embodying the new electrophoresis cell; implementation of techniques for focusing and asymmetry correction; optimization of membranes; and study of problems specific to CPE separation of biological mixtures.

A revised, simplified CPE hydraulic system is planned which greatly reduces the surface area contacting the buffer. This will simplify cleaning and will reduce contamination. A means for varying the hydrostatic pressure in the electrode channels will be provided, to allow controlled slow flow of buffer through the membranes. The performance of microporous membranes, especially in long-term use, will be assessed. An improved sample feed system will pre-equilibrate the pressure at the sample to the pressure in the cell. This will reduce sample loss during startup of sample flow.

We will be able for the first time, with the new cell, to test the asymmetry correction principle. Zonal coatings for this purpose, appropriate to biological samples, will be selected and tested. We will assess the resolution obtainable with the new cell, and effectiveness of collection of closely-resolved components delivered from the expander section. Tables or graphs will be developed which indicate the maximum internal curtain temperature when given buffers are used at various voltage gradients. This will assist in optimizing the separation of heat-labile biological substances.

Work already started in the application and evaluation of carageenan and aparopectin coatings will be completed. Modified or hardened gelatins, gamma globulins, ion-exchange resins and cellulose will be studied as possible coatings of low- or near-zero z.p. Stable reference particles in the intermediate and low z.p. ranges will be developed.

All of the above is in a sense preparation for the principal important objective both here and in space: the optimization of biological separations. Given now an instrument which per se is as effective as the state-of-the-art can provide, we can distinguish and attack the separation problems inherent in the biological materials themselves. Working with tissue cells, leucocytes, sperm cells and bacteria, we will study several effects which at various times have been disturbing. These include aspects of sample conductivity and viscosity, mutual adsorption effects arising from exudates in mixed particles, and particle aggregation. Various corrective measures are available, some tested to an extent in the CPE, others known to us from the published literature. We should be able, accordingly, to develop a body of useful corrective principles and techniques applicable to a wide range of biological materials. This knowledge, we believe, will elevate the CPE technique to a new position of significance among bio-separation methods. Further, it will provide an essential basis of experience for significant biological separations in space.